Marked-up Version of Amendments To Show Changes Made

Additions to the text are indicated by underlining; deletions are indicated by square brackets.

--Recently a polypeptide was isolated, $GABA_BR1a$, that binds radiolabelled $GABA_B$ receptor antagonists in transfected cells (Kaupmann et al. 1997a). The predicted amino acid sequence, as shown in Figures 24A-24D (SEQ ID NO: 48), displays homology with the metabotropic glutamate receptor gene family which includes eight members and a Ca^{++}-sensing receptor. Included in this homology is a large N-terminal domain that contains two lobes with structural similarity to the amino acid binding sites of bacterial proteins. A second polypeptide, $GABA_BR1b$, as shown in Figures 25A-25D (SEQ ID NO: 49), presumably a splice variant, differs from $GABA_BR1a$ in that the N-terminal 147 amino acids are replaced by 18 different residues in the predicted mature protein after signal peptide cleavage. Transcripts for both $GABA_BR1s$ are abundant and widely distributed in the rat brain. There appear to be differences in the localization of the splice variants in discrete regions of the brain, suggesting that their expression is differentially regulated (Bischoff et al. 1997).--

--208. (Twice Amended) A process for determining whether a chemical compound is an agonist of a mammalian $GABA_BR1/R2$ receptor which comprises contacting cells containing nucleic acid encoding, and expressing on their cell surface, the $GABA_BR1/R2$ receptor, wherein such cells prior to being transfected with such nucleic acid do not express the $GABA_BR1/R2$ receptor, with the compound under conditions permitting the activation of the $GABA_BR1/R2$ receptor, and detecting an increase in activity of the $GABA_BR1/R2$ receptor, so as to thereby determine whether the compound is

an agonist of a $GABA_BR1/R2$ receptor, wherein the mammalian $GABA_BR1/R2$ receptor comprises a $GABA_BR1$ polypeptide and a $GABA_BR2$ polypeptide, which $GABA_BR1$ polypeptide has an amino acid sequence identical to the amino acid sequence shown in Figures 24A-24D (SEQ ID NO: 48) or Figures 25A-25D (SEQ ID NO: 49), and which $GABA_BR2$ polypeptide has an amino acid sequence (a) identical to the amino acid sequence shown in Figures 4A-4D (SEQ ID NO: 4) or Figures 23A-23D (SEQ ID NO: 47), or (b) encoded by a nucleic acid sequence identical to the receptor-encoding nucleic acid sequence contained in plasmid pEXJT3T7-hGABAB2 (ATCC Accession No. 203515) or in plasmid BO-55 (ATCC Accession No. 209104) [, or (c) which varies from one of the amino acid sequences of (a) or (b) in terms of the identity or location of an amino acid residue without changing the properties of the $GABA_BR2$ polypeptide].--

--213. (Twice Amended) A process for determining whether a chemical compound activates a mammalian $GABA_BR1/R2$ receptor, which comprises contacting cells producing a second messenger response and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells prior to being transfected with such nucleic acid do not express the $GABA_BR1/R2$ receptor, with the chemical compound under conditions suitable for activation of the $GABA_BR1/R2$ receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in the second messenger response in the presence of the chemical compound indicating that the compound activates the $GABA_BR1/R2$ receptor, wherein the mammalian $GABA_BR1/R2$ receptor comprises a $GABA_BR1$ polypeptide and a $GABA_BR2$ polypeptide, which $GABA_BR1$

polypeptide has an amino acid sequence identical to the amino acid sequence shown in Figures 24A-24D (SEQ ID NO: 48) or Figures 25A-25D (SEQ ID NO: 49), and which $GABA_BR2$ polypeptide has an amino acid sequence (a) identical to the amino acid sequence shown in Figures 4A-4D (SEQ ID NO: 4) or Figures 23A-23D (SEQ ID NO: 47), or (b) encoded by a nucleic acid sequence identical to the receptor-encoding nucleic acid sequence contained in plasmid pEXJT3T7-hGABAB2 (ATCC Accession No. 203515) or in plasmid BO-55 (ATCC Accession No. 209104) [, or (c) which varies from one of the amino acid sequences of (a) or (b) in terms of the identity or location of an amino acid residue without changing the properties of the $GABA_BR2$ polypeptide].--

--224. (Twice Amended) A method of screening a plurality of chemical compounds not known to activate a mammalian $GABA_BR1/R2$ receptor to identify a compound which activates the $GABA_BR1/R2$ receptor, wherein the mammalian $GABA_BR1/R2$ receptor comprises a $GABA_BR1$ polypeptide and a $GABA_BR2$ polypeptide, which $GABA_BR1$ polypeptide has an amino acid sequence identical to the amino acid sequence shown in Figures 24A-24D (SEQ ID NO: 48) or Figures 25A-25D (SEQ ID NO: 49), and which $GABA_BR2$ polypeptide has an amino acid sequence (a) identical to the amino acid sequence shown in Figures 4A-4D (SEQ ID NO: 4) or Figures 23A-23D (SEQ ID NO: 47), or (b) encoded by a nucleic acid sequence identical to the receptor-encoding nucleic acid sequence contained in plasmid pEXJT3T7-hGABAB2 (ATCC Accession No. 203515) or in plasmid BO-55 (ATCC Accession No. 209104) [, or (c) which varies from one of the amino acid sequences of (a) or (b) in terms of the identity or location of an

amino acid residue without changing the properties of the $GABA_BR2$ polypeptide,] which comprises:

(a) contacting cells containing nucleic acid encoding, and expressing on their cell surface, the $GABA_BR1/R2$ receptor, wherein such cells prior to being transfected with such nucleic acid do not express the $GABA_BR1/R2$ receptor, with the plurality of compounds not known to activate the $GABA_BR1/R2$ receptor, under conditions permitting activation of the $GABA_BR1/R2$ receptor;

(b) determining whether the activity of the $GABA_BR1/R2$ receptor is increased in the presence of the compounds, and if it is increased;

(c) separately determining whether the activation of the $GABA_BR1/R2$ receptor is increased by each compound included in the plurality of compounds, so as to thereby identify the compound or compounds present in such a plurality of compounds which activates the $GABA_BR1/R2$ receptor.--

--231. (Twice Amended) A process for determining whether a chemical compound is an agonist of a mammalian $GABA_BR1/R2$ receptor, which comprises preparing a membrane fraction from cells which comprise nucleic acid encoding, and expressing on their cell surface, the $GABA_BR1/R2$ receptor, wherein such cells prior to being transfected with such nucleic acid do not express the $GABA_BR1/R2$ receptor, separately contacting the membrane fraction with both the chemical compound and GTPγS, and with only GTPγS, under conditions permitting the activation of the $GABA_BR1/R2$ receptor, and detecting GTPγS binding to

the membrane fraction, an increase in GTPγS binding in the presence of the compound indicating that the chemical compound activates the $GABA_BR1/R2$ receptor, wherein the mammalian $GABA_BR1/R2$ receptor comprises a $GABA_BR1$ polypeptide and a $GABA_BR2$ polypeptide, which $GABA_BR1$ polypeptide has an amino acid sequence identical to the amino acid sequence shown in Figures 24A-24D (SEQ ID NO: 48) or Figures 25A-25D (SEQ ID NO: 49), and which $GABA_BR2$ polypeptide has an amino acid sequence (a) identical to the amino acid sequence shown in Figures 4A-4D (SEQ ID NO: 4) or Figures 23A-23D (SEQ ID NO: 47), or (b) encoded by a nucleic acid sequence identical to the receptor-encoding nucleic acid sequence contained in plasmid pEXJT3T7-hGABAB2 (ATCC Accession No. 203515) or in plasmid BO-55 (ATCC Accession No. 209104) [, or (c) which varies from one of the amino acid sequences of (a) or (b) in terms of the identity or location of an amino acid residue without changing the properties of the $GABA_BR2$ polypeptide].--